Supplementary Online Material

Serotonin Uptake is Largely Mediated by Platelets versus Lymphocytes in Peripheral Blood Cells

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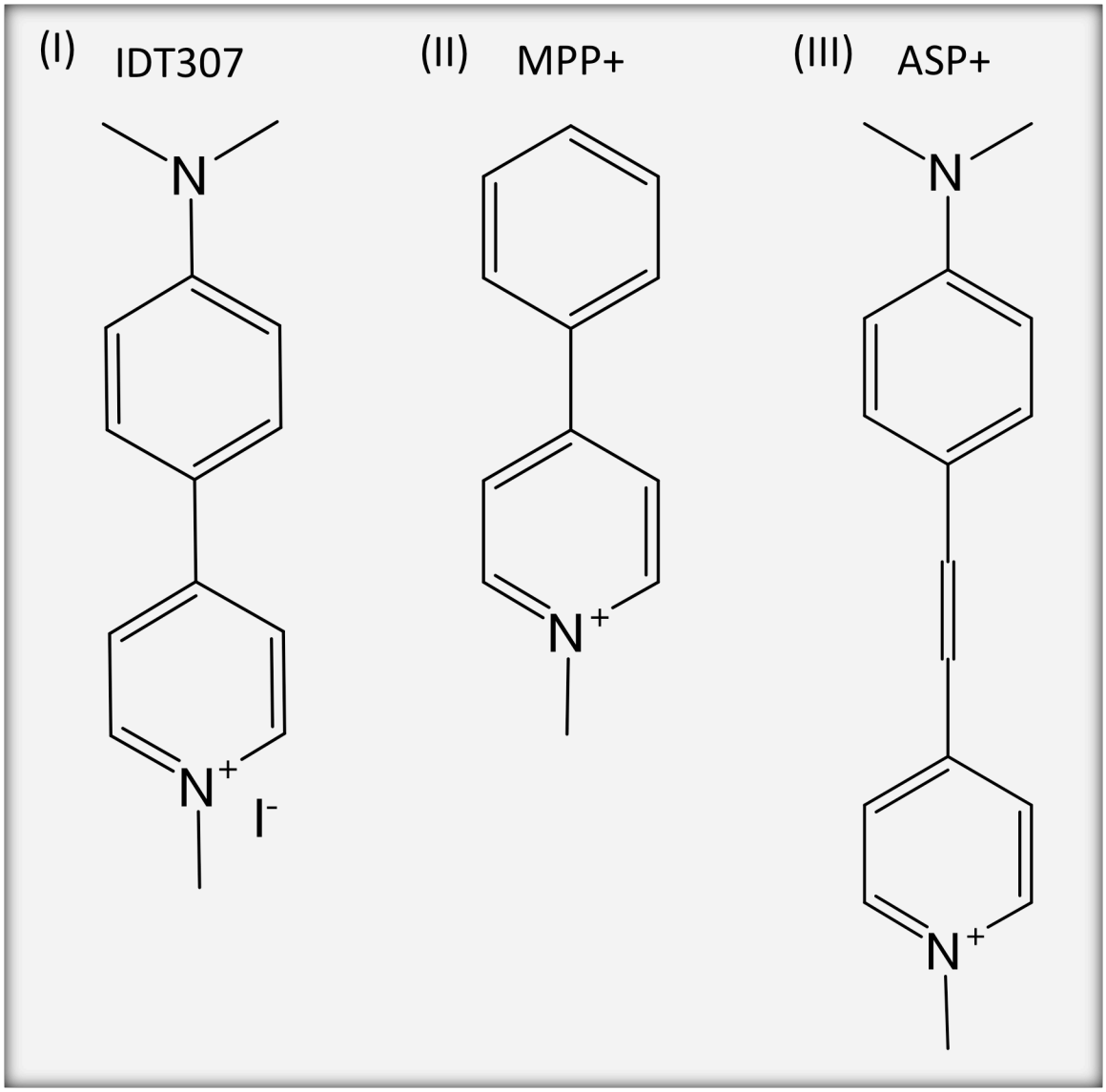

Figure S1. Chemical structures for (I) IDT307, (II) MPP+, and (III) ASP+. IDT307 and ASP+ are analogs of the nonfluorescent dopaminergic neurotoxin MPP+, which is a DAT substrate. IDT307 (US Patent #7947255) is a substrate for SERT, DAT, and NET. Similar to ASP+ (*1*), IDT307 will fluoresce after being taken up into the intracellular environment and intercalating with biomolecules so as to adopt a planar configuration.

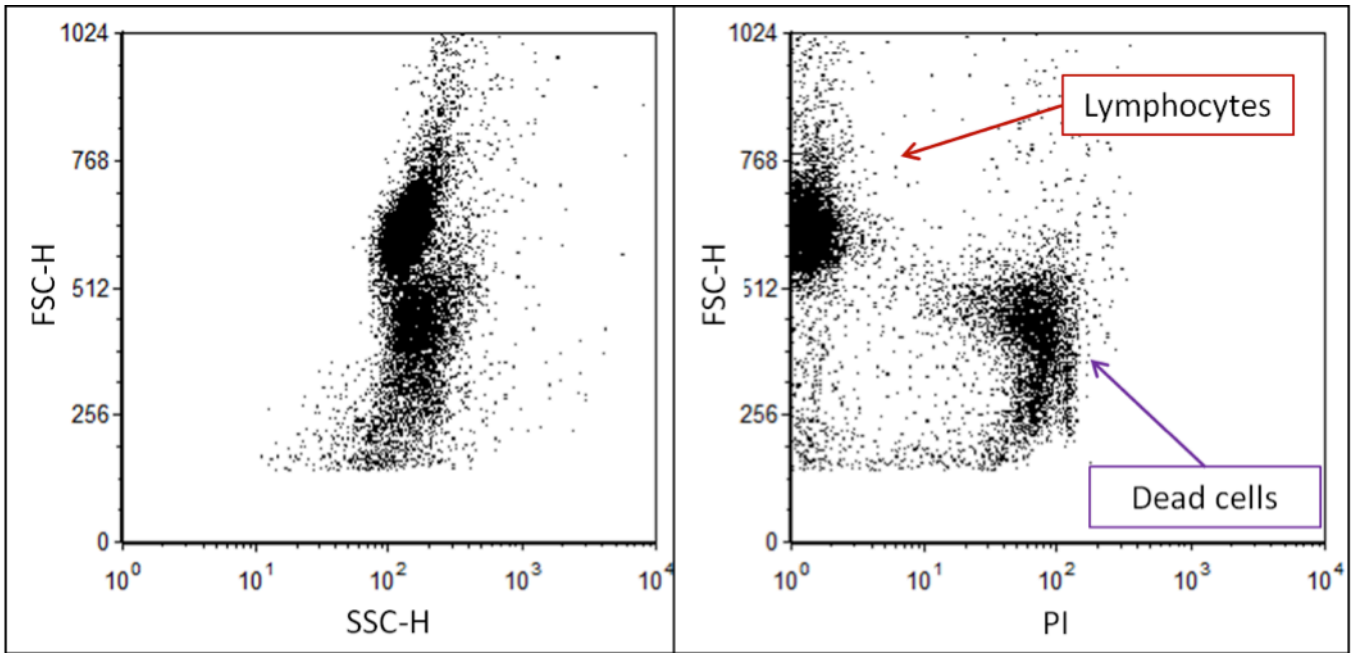

Figure S2. Identification of mouse lymphocytes by magnetic bead separation. (Left) After magnetic bead isolation of mouse B lymphocytes, two cell populations are visible in the side scatter (SSC-H) versus forward scatter (FSC-H) plot. **(Right)** Propidium iodide staining causes fluorescence associated with dead cells (x-axis) to increase (lower population only). The upper population does not show increased fluorescence and is identified as viable/live B lymphocytes. This information was used to gate live B lymphocytes isolated directly from spleen in Figure 3.

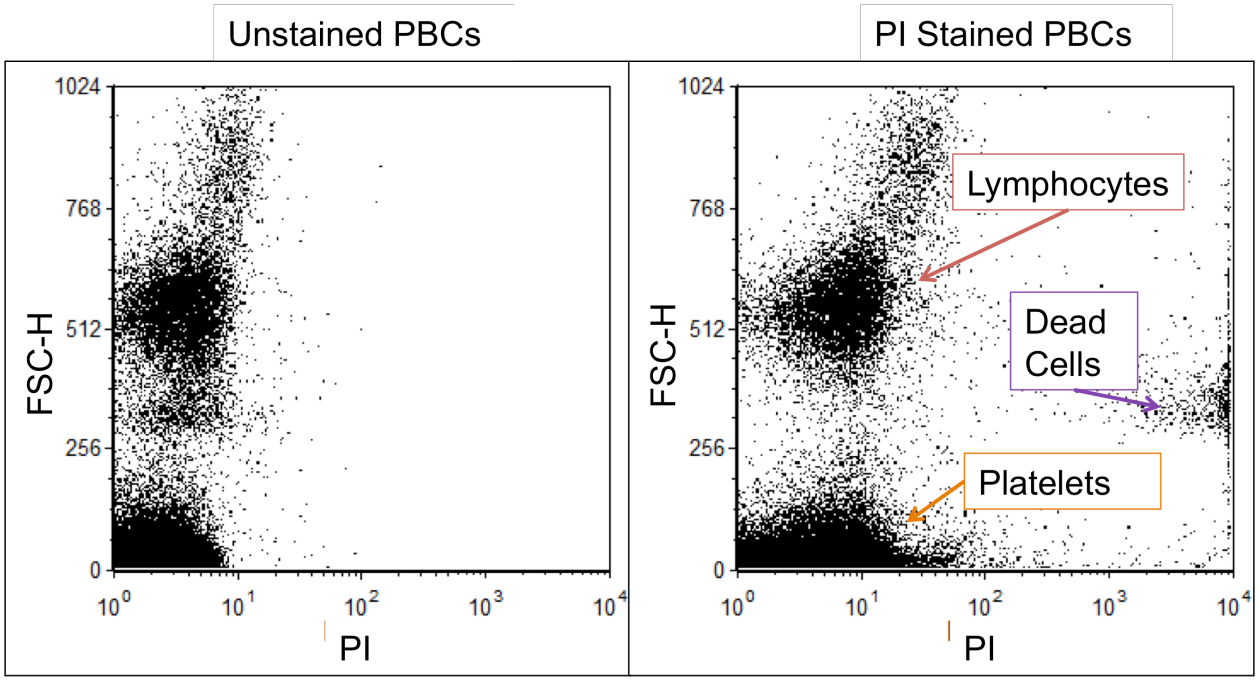

Figure S3. Propidium iodide (PI) staining to identify dead cells in human cell samples. (Left) Forward-scatter (FSC-H) *vs* PI fluorescence (excitation 488 nm; emission 585 nm) in peripheral blood cells in the absence of PI. Cell membranes of live cells are impermeable to PI. **(Right)** In the presence of PI, dead cells show fluorescence associated with PI due to DNA intercalation. All samples for IDT307 uptake were analyzed in parallel with similar samples stained with PI to enable fluorescence associated with dead cells to be subtracted prior to the analysis of IDT307 fluorescence.

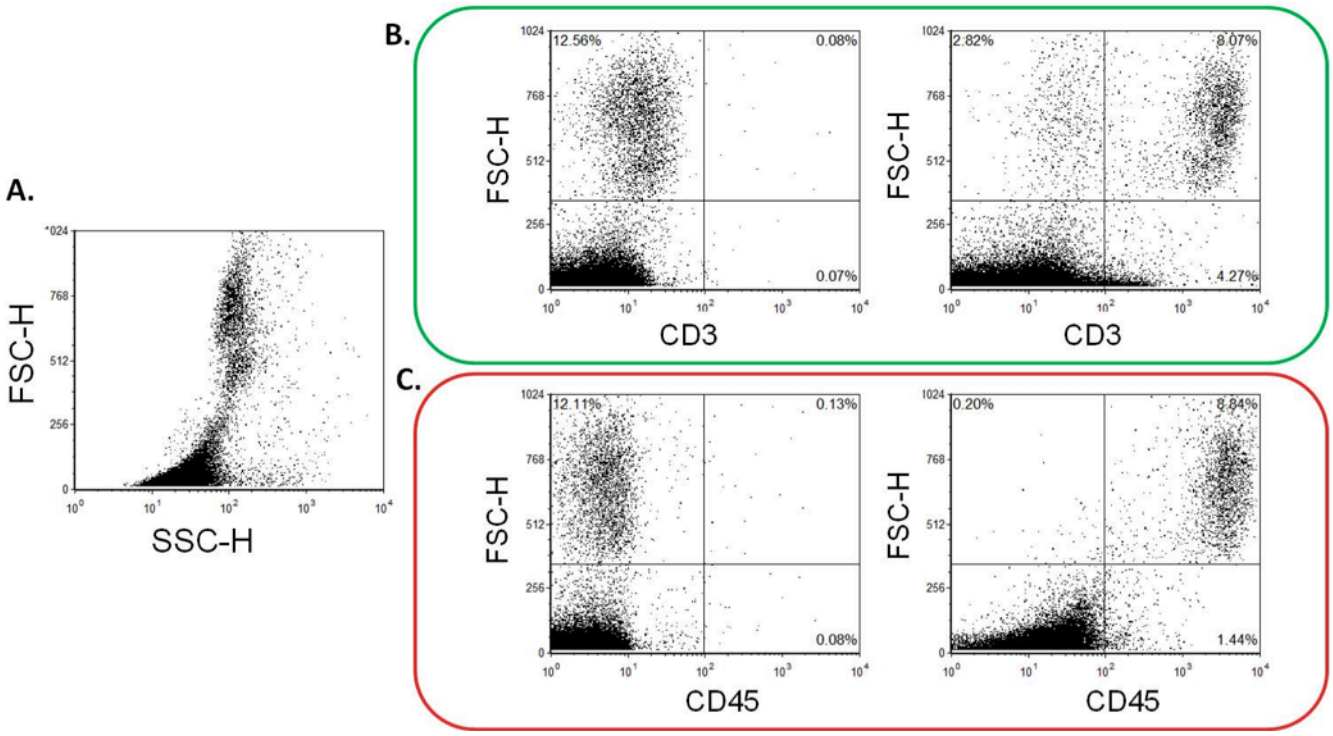

Figure S4. Identification of lymphocytes by cell-surface specific antigens in rhesus peripheral blood cells. A) Representative forward-scatter (FSC-H) versus side-scatter (SSC-H) plot of rhesus PBCs. **B)** Rhesus lymphocytes (top quadrants), but not platelets (bottom quadrants), exhibit 100× greater fluorescence in the presence of fluorescently-labeled CD3 antibodies (CD3) or **C)** CD45 antibodies (CD45). CD3 (T-cells) and CD45 (leukocytes) are cell-surface antigens that are specific to lymphocyte cell types versus platelets.

Supplementary Methods

Magnetic-bead separation of mouse splenocytes. Mouse B-lymphocytes were isolated using the MACS B-cell Isolation Kit (Miltenyi Biotec, Auburn, CA) as per the manufacturer's instructions. Spleens were obtained as stated in the main text Methods section. After splenocytes were isolated and counted, they were resuspended in 40 µL of PBS per every 10^7 cells. Next, 10 µL of biotin-antibody cocktail were added for every 10^7 cells and cells were incubated with antibody for 15 min at 4 °C. Afterwards, 30 µL of additional buffer and 20 µL of anti-biotin MicroBeads were added per 10^7 cells. Samples were incubated for an additional 15 min, after which 1-2 ml of PBS was added and cells were centrifuged at 500 × g for 10 min. Cells were then suspended at a density of 10^8 cells/500 µL buffer and run through a magnetic MACS separation column on a MACS separator. Using this kit, B-lymphocytes are unlabeled and allowed to pass through the column for collection, whereas all other biotin-antibody-labeled immune cells remain bound to the column via the conjugated magnetic beads.

Antibody staining of rhesus lymphocytes. Alexa Fluor 488-conjugated CD3 antibodies (product no. 447705) and PE- (R-phycoerythrin) conjugated CD45 antibodies (product no. 552833) (BD Biosciences, San Jose, California) were used to identify lymphocyte populations. Peripheral blood cells were thawed and resuspended (10^6 cells) in 100 µL of assay buffer. Each sample was divided to include a control sample that contained no antibody, a CD-3-only sample, and a CD45-only sample. Antibodies were added to samples and allowed to incubate at room temperature for 25 min. Afterward, samples were centrifuged at 500 × g for 10 min and resuspended in 500 µL fresh assay buffer. Samples were analyzed by flow cytometry (excitation wavelength 488 nm; emission wavelengths 520 nm for CD3-Alexa Fluor 488 and 588 nm for CD45-PE).

References

1. Mason, J. N., Farmer, H., Tomlinson, I. D., Schwartz, J. W., Savchenko, V., DeFelice, L. J., Rosenthal, S. J., and Blakely, R. D. (2005) Novel fluorescence-based approaches for the study of biogenic amine transporter localization, activity, and regulation, *J Neurosci Methods 143*, 3-25.